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Translation No. [redacted]

Author: Hans Timmel, Pathological Institute of The Martin Luther University (Pathologischen Institut der Martin-Luther Universitat) Halle-Wittenberg, Germany

Title: On the localization of the milker's node virus in the tissues (Zur Lokalisation des Melkerknoten-Virus im Gewebe).

Journal: Acta Biologica Medica Germanica 9: 79-82 (1962).

October 1968

SUMMARY

As far as the localization of the milker's node virus in tissue is concerned, the author reports for the first time on findings obtained by means of ultra-thin slices. Two cases of milker's nodes so far examined by the electron-optical method show the following distribution of viruses: In the corium no viruses can be traced, not even within the region of granulomatous changes. In the stratum basale, the enlarged epidermis does not show any elementary bodies. In the stratum spinosum, one finds viruses increasing in number towards the outer layers and which are most densely concentrated in the layer neighboring the stratum corneum. In the stratum corneum, viruses were traced as mere shadows. Annular viruses are located in the inner strata, while typically square-shaped (quadrangular) forms of viruses are prevailing in the outer layers of the stratum spinosum and in the stratum corneum. The morphological substrate explains the healing of the milker's nodes without leaving scars. ( )

For the first light-optical demonstration of the agent of milker's nodes[2], we thank Lipschutz (1) (= Strongyloplasma paravaccinae).

Of the numerous subsequent investigations, only a few could report on positive results for the detection of Strongyloplasma paravaccinae (2)-(6).

Finally, in recent times, Puntigam and Orth (7) as well as Nasemann and Deubner (4) and Bauer (6) have studied the electron microscopic demonstration of the virus. The latter established the quadrangular (square-shaped) structure and enzymatic lability of the virus. As a result, they one again confirmed the morphological similarity of this virus to the pox agent already described by Lipschütz (1).

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[2] virus of milker's nodes sensu strictiori (Berger(8)).

All of the above-mentioned investigations, however, were carried out entirely on smeared preparations, that is, on the fluids of pressed-out tissues.

With reference to the localization of the virus in the milker's node, insufficient information is found in the literature.

Lipschütz (1) was able to demonstrate "numerous elementary bodies" in inclusion-containing cells which showed reticular degeneration. Simultaneously, he assumed that the agent had "an effect on the endothelium of the blood vessels of the skin" since he was able to demonstrate from the histological results an abundantly vascular, granulomatotic inflammation in the corium. Moreover, Nasemann and Bauer (6) in their investigations demonstrated elementary bodies in an epidermal layer (see their Fig. 2).

In the following is a report using ultra-thin sections for studying the localization of the virus of milker's node in the tissues.

MATERIAL AND METHODS

Freshly excised milker's node tissues from two patients (30 years old ♂ ; 45 years old ♀) of the local University Skin Clinic were available to us as starting material[1].

The tissues were immediately cut into pieces less than 1 mm in length and fixed for 2 to 3 hours in osmic acidsolution buffered at pH 7.2 with veronal-acetate. Negative contrast staining was carried out in 0.5 % phosphotungestic acid in 70 % alcohol or acetone for 30 minutes. The imbedding was carried out in the usual manner in methacrylic acid ester (9) (mixture ratio of butyl ester to methyl ester = 9:1) and in Vestopal-W (10).

The thin sections were prepared with an ultra-microtome by the method of Niklowitz. For evaluation, an Elmi-D2 electron microscope (VEB Zeiss, Jena)

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[1] Dr. Krinitz, Chief Physician at the University Skin Clinic is thanked at this time for his completely appreciated cooperation.

was employed. Photography material: electron-plated paper from the VEB Film Company, Agfa-Wolfen.

Results in The Corium

In the corium, viral particles were found neither in the stratum reticular nor in the stratum papillar. The electron microscopic picture confirmed the light optical details of the so-called viral granuloma. Especially impressive was the local sub-epidermal edema with untwisting of tissue constituents as well as the abundance of vesicles.

Results in The Epidermis

Stratum basale: The stratum basale of the epidermis showed well contained details of an intact basal membrane. The epithelial cells had inconspicous cell nucleic and contained cytoplasmic internal structures (see Fig. 4). Viruses could not be detected here.

Stratum spinosum: Numerous viruses were found in the cells of the stratum spinosum. Both quadrangular (sqaure-shaped) viruses and annular (ring-shaped) preliminary stages could be detected in this layer. Certain regularities were shown in their distribution. Annular developmental stages of the virus with electron-dense inner structures (Fig. 2) could be shown to prevail in the inner layers of the stratum spinosum, which showed less degenerative alterations of cell nuclei and cytoplasm than did the cells near the epidermis. They were mostly located in dense groups near to the nucleic in a finely granular, osmiophilic substance, the viroplasma (11). In addition, individual, mature, quadrangular viruses are often found.

In the exterior cells of the stratum spinosum lying near the epidermis as well as in the deeper lying cells, in which projecting degenerative alterations can be found, quadrangular viruses are usually the rule. These exhibit an

inner structure similar to that of the viruses of the pox group (2) (Fig. 3; Fig. 3a). In the exterior cell layers, annular developmental stages of the virus are found mostly only in association with very electron-dense cytoplasmic regions. This corresponds to the light-optical "inclusions".

Stratum corneum: At the point of transition of the stratum spinosum to the widespread stratum corneum, the viruses are the most abundant (Fig. 3). They can also be detected in abundant numbers in the stratum corneum even with the epidermal layer which does not appear to have a great deal of structure under the electron microscope (Fig. 4).

Occasionally, the viruses are found exposed between epidermal lamellae. However, they are usually observed intracellularly only. Their presence decreases in the surface epidermal layers.

DISCUSSION

The investigations indicate a pronounced epitheliotropy for the milker's node virus. In the regions of the vascular-rich granuloma of the corium one is able to detect neither intracellular nor extracellular particulate organizational stages of the milker's node virus. The findings on the localization of the virus have been summarized (Fig. 5). It is of significance to note the predominance of annular developmental stages of the virus in the inner layers of the stratum spinosum and the accumulation of quadrangular viruses in the exterior layers of the diffuse epidermis.

The absence of virus and of degenerative cell alterations in the stratum basale provides a clear morphological explanation for the healing of infections without scars.

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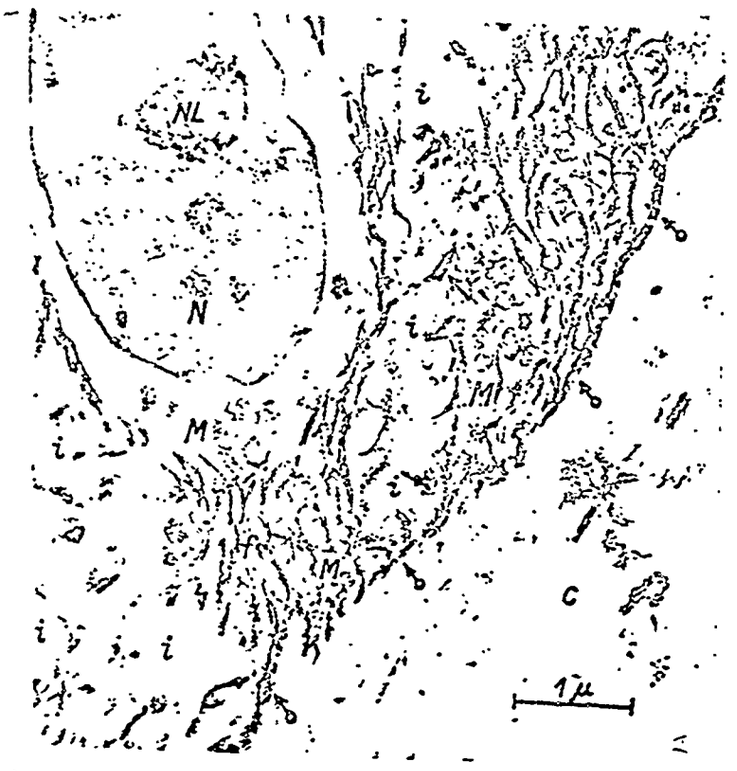

Fig. 1. Section of the stratum basale and the edematous corium (c). N= nucleus of a basal epithelial cell; Nl= nucleolus; i = intercellular cleavage space; f= tonofibril bundle; m = mitochondria; ↗ marks a desmosome; intact basal membrane of ♂ .

Fig. 2. Section of a cell from the inner stratum spinosum. Numerous annular viruses (Vr) are found and only isolated quadrangular elementary bodies (Vq). The section of the picture at the bottom right is strongly enlarged. i = intercellular cleavage space; f = bundle of tonofibrils in which keratohylin is already imbedded; K = keratohylin body; cm = cytoplasmic membrane.

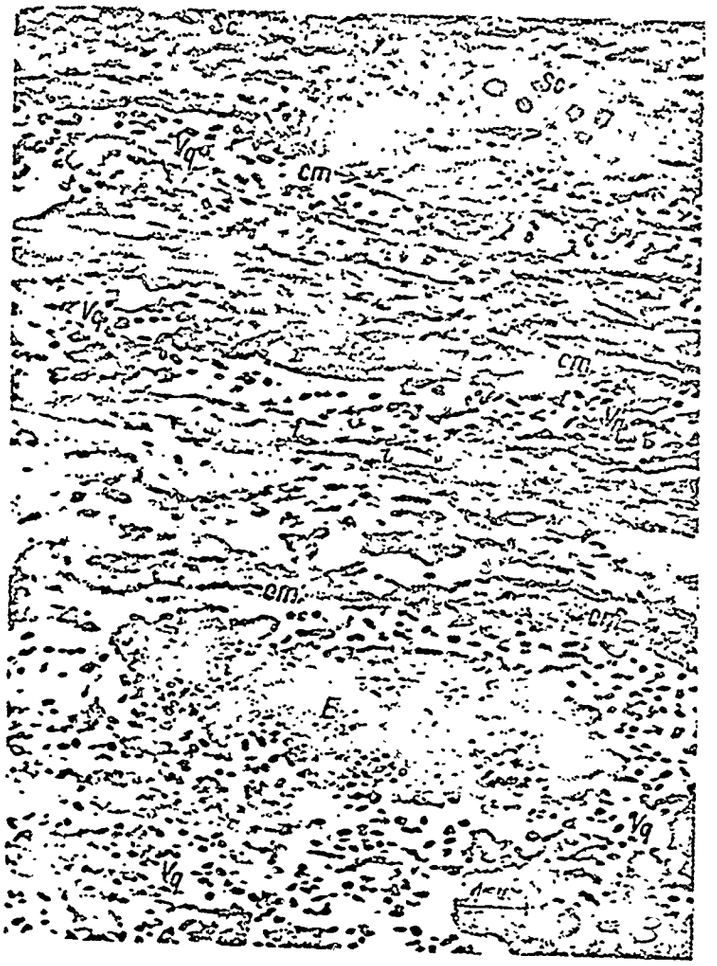

Fig. 3. Upper stratum spinosum with numerous flattened cells and transition to the stratum corneum (Sc). Quadrangular viruses (Vq) dominate the picture. At E is olcated an "inclusion" which is visable under the light microscope and contains numerous elementary bodies clumped together. cm = cytoplasmic membrane; between the arrows ↗ is found the narrow projection of an epithelial cell; i = intercellular cleavage space.

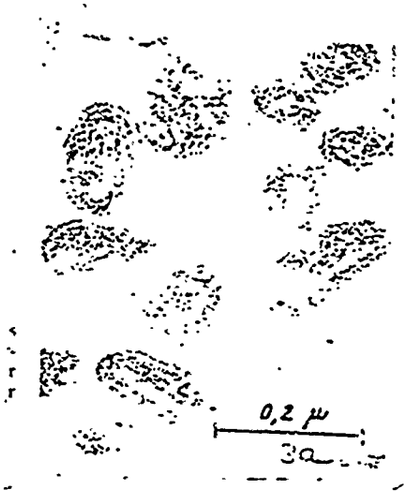

Fig. 3a. Magnification of a section from the stratum spinosum. Quadrangular virus with an internal structure similar to the viruses of the pox group.

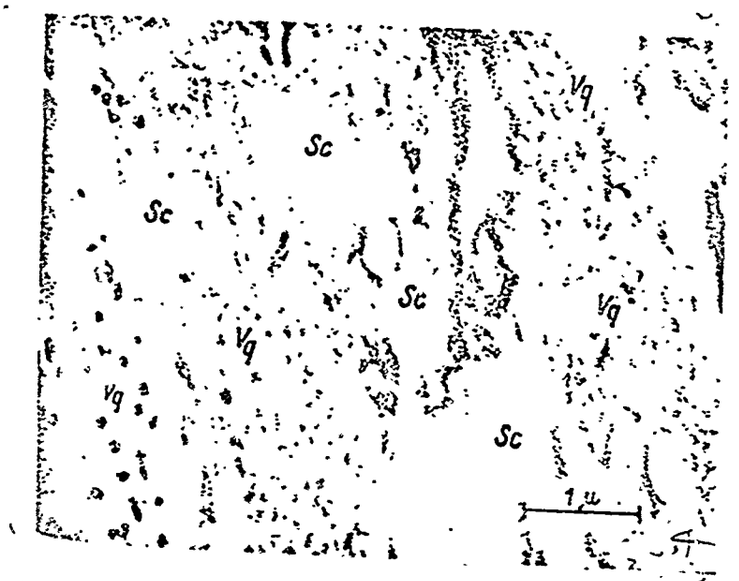

Fig. 4. Section from the widespread stratum corneum. In the callous layer, which is easily seen under the electron microscope, are found numerous shaded viruses (Vq) with typical exterior shapes.

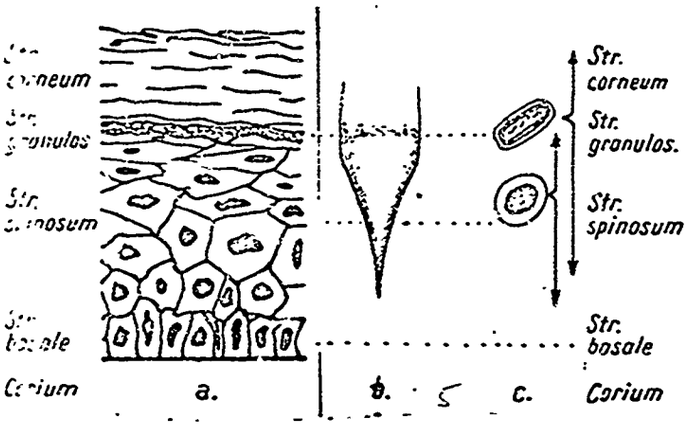

Fig. 5. The localization of the milker's node virus. (a) Layers of the epidermis. The stratum granulosum is usually not demonstrable in milker's nodes (see (5)). (b) The density of the virus infection is symbolized by the width of the dark column. The lightening of the upper section characterizes occasional detection of the virus. (c) The developmental forms of the virus in relation to the epithelial layers.